# Cannibalism in *Amoeba vespertilio* (Penard).

By

**Geoffrey Lapage, M.Sc., M.B.,**

Lecturer in Zoology, The Victoria University of Manchester.

With Plates 28, 29, and 3 Text-figures.

## 1. Material and Methods.

Towards the end of July 1920 an old hay infusion, which had been made some ten years previously and had been left untouched in the laboratory since then, was examined to find out what organisms it still contained. Among a fairly abundant fauna, which included Ciliata and some Flagellata, a good supply of small amoebae was obtained from the bottom deposit.

By transferring portions of this bottom deposit to Petri dishes and adding tap-water, these amoebae were cultivated. Although some of the cultures failed, others throve well, especially those in which the amoebae were feeding on the layer of small diatoms which quickly spread over the bottom of the dish and were present also in the clumps of vegetable débris.

From time to time aquarium water and boiled hay infusion were added to replace the loss of fluid by evaporation.

The amoebae were examined on slides with and without cover-glasses ; but this method was soon abandoned in favour of hanging drops, made in the following way :

A glass ring, vaselined on both surfaces, was placed upon an ordinary slide. A cover-glass, upon which a drop of the culture fluid from the bottom of the culture had been placed, was inverted, lowered upon the glass ring and then pressed down, so that the drop hung in a sealed chamber. A small drop of water was placed on the bottom of the chamber before it was closed, as an additional precaution against evaporation. These preparations were similar to those used for the study of Helkestimastix (Woodcock and Lapage, **31**) and in them the amoebae could be observed for several days. It was found that, after a day or two, all the organisms in the drops, and especially Ciliata such as Paramoecium bursaria, became very sluggish; but they could be readily revived by lifting the cover-glass for a few minutes and replacing it again. The renewal of the air in the chamber, effected in this way, had a remarkably invigorating effect upon the organisms, the Paramoecium bursaria, for example, immediately resuming their normal lively activity. The method had the additional advantage that the organisms under observation could be fixed at any desired moment, by simply removing the cover-glass, spreading out the drop upon it, after removing the adherent vaseline, and then dropping the film on to the surface of a dish of fixative.

Permanent preparations were constantly made in this manner. In addition amoebae were daily taken from the cultures and fixed upon albumenized slides, the culture fluid being spread out in a thin film before the fixative was added.

The fixative used was that introduced by Dr. H. M. Woodcock and was made up of two parts of a saturated solution of corrosive sublimate in water to one part of absolute alcohol with glacial acetic acid in the proportion of 5 per cent. Most of the slides were stained by Dobell's alcoholic modification of Heidenhain's iron haematoxylin method (Dobell, **8**). This method, though in some respects inferior to the watery iron haematoxylin method, gave very good results. It has the double advantage over the watery method of being quicker and of avoiding the treatment of the preparation with water or the lower grades of alcohol, in which many organisms, unless previously hardened overnight in 70 per cent. or 90 per cent. alcohol, are frequently washed off. It is undoubtedly a very useful and reliable method for staining all kinds of

Protozoa. Other stains used were Heidenhain's watery iron haematoxylin and Delafield's haematoxylin. A counterstain was not used, since it is quite unnecessary after these stains and, in the opinion of the writer, tends to obscure, rather than to improve, the results. Both Bausch and Lomb and Zeiss microscopes were used, the ordinary high power dry lens being sufficient for most of the observations on the living objects; but, when higher magnification was needed, a Zeiss apochromatic oil immersion was used.

## 2. Characters of Amoeba vespertilio.

Considerable difficulty was experienced in the identification of the amoebae present in the cultures. It is not my intention to enter here into this vexed question; but it is necessary to record the opinion that species of amoebae established upon descriptions of their external characters alone, without a prolonged study of them under all conditions and a knowledge of their nuclear apparatus and life-history, supported by the evidence of stained preparations, must be regarded as provisional only.

Until such detailed knowledge is available, however, the existing data must be utilized; and, when I say that the amoeba which forms the subject of this paper corresponds with that described by Doflein (**9**) and Penard (**21**) as *Amoeba vespertilio*, it should be understood that I do not necessarily regard *Amoeba vespertilio* as a true species.

The account and figures of this amoeba given by Doflein (**9**) are so excellent, and my own observations upon it confirm his so exactly, that it is unnecessary for me to give here more than a summary of its distinctive characters.

*Amoeba vespertilio* is a small amoeba, showing a well-marked contrast between clear ectoplasm and granular endoplasm, and is, when healthy, very active. Its pseudopodia are typically branched, with pointed ends, and are composed mostly of ectoplasm. When the amoeba is creeping along a substratum, it assumes a very characteristic shape, resembling that of a bat's wing or of a duck's foot. The form is, however, very variable and, under certain chemical and physical conditions, star-shaped and other forms occur.

The nucleus is vesicular, with a well-marked endosome, which stains deeply and shows, in preparations which have been suitably differentiated, a well-marked meshwork structure (Pl. 28, fig. 1). This endosome is surrounded by a clear halo in which no structure can be made out, and this clear area does not appear to be separated from the endoplasm of the amoeba by a definite nuclear membrane. The area of endoplasm immediately surrounding the nucleus stains, however, more deeply than the rest of the endoplasm, an effect which is due to the heaping up, as it were, in this region, of the fine granules of deeply-staining matter which are distributed throughout the endoplasm on the strands of its meshwork.

The size of the amoeba varies considerably. Doflein (**9**) gives the size of the motile creeping forms as being 220–250 $\mu$ long by 40–60 $\mu$ broad, whilst the star-shaped forms measured from 60–150 $\mu$, according to the length of their pseudopodia. He says that the nucleus varies from 10–15 $\mu$ in diameter and the endosome from 7–10 $\mu$. The amoebae in my cultures were rather smaller than this, the motile forms reaching 200 $\mu$ long and sometimes rather longer, when the pseudopodia were well extended; but the majority of both motile and star-shaped forms varied between 60–100 $\mu$ in diameter. The nucleus measured from 7–9 $\mu$ in diameter and the endosome from 4–7 $\mu$. It should be mentioned, however, that these measurements were made upon stained preparations in which some shrinkage may have occurred.

The endoplasm usually contains numerous vacuoles as well as abundant granules. One or more contractile vacuoles are present. Penard (**21**) states that generally there is only one, but that two or three are often present, one of which seems to be the principal one, and that there is almost always a great number of vacuoles distributed here and there, which appear and disappear as if they played the part of contractile vacuoles. I have also found that the presence of several contractile vacuoles is a frequent feature of the amoebae in cultures, but amoebae with only one contractile vacuole were at least as common.

Doflein (**9**) placed some of his amoebae in a culture which contained Frontonia leucas which were full of green zoochlorellae. The amoebae fed upon the 'remains' of the Frontonias and themselves became infected with zoochlorellae. A similar infection occurred in come of my cultures also, the zoochlorellae being acquired in this case apparently from Paramoecium bursaria. These zoochlorella-infected amoebae were not, however, used for any of the observations described below and there is no evidence that cannibalism occurred in them.

The cultures also contained other small amoebae, which were definitely different in external appearance from the Amoeba vespertilio, and which remained so. As far as I was able to judge from external characters only, these small amoebae were of the Amoeba limax or Vahlkampfia type. Their average size was 28–30 $\mu$ long by 6–8 $\mu$ broad: but their length varied from 20–50 $\mu$, and their breadth from 4–12 $\mu$. They possessed a vesicular nucleus, similar in structure to that of Amoeba vespertilio, its diameter being 5–6 $\mu$, while the diameter of its endosome was 3 $\mu$. These amoebae were present in large numbers in some of the cultures, especially in the later stages of the work.

A few amoebae, with a diaphanous appearance, rather larger than the A. limax and without the slug-like form which is characteristic of the latter, were not identified. They may have been either large A. limax forms or small examples of Amoeba vespertilio, or they may have belonged to another species altogether.

Other Protozoa present in the cultures included Paramoecium bursaria, Pleuronema chrysalis, and numbers of small Flagellata. No Thecamoebida were ever seen.

### 3. Observations on the Spheres.

The amoebae had not been long under observation before attention was arrested by certain remarkable inclusions which many of them contained.

These were nucleated, spherical bodies, with a sharply-defined outline, whose protoplasm resembled that of the amoebae themselves. They would, indeed, have been almost indistinguishable from the amoebae containing them had they not been in some cases enclosed in a very obvious vacuole, the margin of which was very distinct. Between the enclosed body and the margin of the vacuole was a space, varying in extent in different cases (cf. Pl. 28, figs. 2 and 5; Pl. 29, figs. 9, 10, and 11), which was pinkish in colour and presumably contained fluid.

The diameter of the spheres varied between 8–46·5 $\mu$, both these figures representing extreme sizes. The majority of them varied between 20–26 $\mu$. They were very distinct and well-marked objects, much larger than the ordinary food vacuoles. In certain positions of the amoebae, however, when the endoplasm was packed with food or when the protoplasm, in the course of its streaming, became heaped up over the sphere, the latter became very indistinct. This was especially the case in the rare examples in which the vacuole round the sphere was narrow. It was then difficult to determine the exact line of demarcation between the enclosed sphere and the surrounding protoplasm. On such examples it is quite possible for an inexperienced worker to mistake the spheres, in spite of their large size, for the nucleus, a point to which we shall return later (cf. below, p. 690).

In stained preparations the spheres showed a typical vesicular nucleus, exactly similar in structure to the nucleus of the amoeba itself, consisting, that is to say, of a central endosome with a meshwork structure, surrounded by a clear halo, free from chromatin and apparently structureless. Here again, as in the amoeba, no definite nuclear membrane could be made out. The whole nucleus of the sphere, including the clear halo round the endosome, measured from 6–8 $\mu$, and the endosome itself from 4–5 $\mu$.

Since the measurements were made from stained preparations, in which some shrinkage may have occurred, the actual size may have been rather larger than this, although very little difference was observed between the sizes of the nuclei and the endosomes of amoebae and spheres of the same size: but, of course, the bigger spheres showed bigger nuclei than the smaller ones. A comparison of these measurements of the nuclei of the spheres with those of the nuclei of the amoebae was very striking:

| | | | |
|---|---|---|---|
| Diameter of the amoeba | 60–130 μ | Diameter of sphere . | 20–26 μ |
| Diameter of nucleus of amoeba . . . | 7–9 μ | Diameter of nucleus of sphere . . . . | 6–8 μ |
| Diameter of endosome of amoeba . . . | 4–7 μ | Diameter of endosome of sphere . . . . | 4–5 μ |

The correspondence was very remarkable, especially when it was noted that the sphere scarcely differed in any respect, except in shape, from the amoeba and was almost indistinguishable from the rounded-off forms of the latter.

While it was inside the vacuole, the sphere was never seen to move in any way by its own efforts. It was not ciliated nor flagellated, nor did it put out pseudopodia, but maintained, in most cases, a perfectly even spherical contour, although a few cases were seen in which its outline was oval or even irregular (Pl. 29, figs. 8 and 10). The spheres were sometimes rolled over and over in the vacuoles by the streaming movements of the protoplasm, in which case the whole vacuole probably rolled about as a whole. But, in one instance, when the streams of protoplasm were very active along the sides of the vacuole, the enclosed sphere was seen to rotate in the opposite direction.

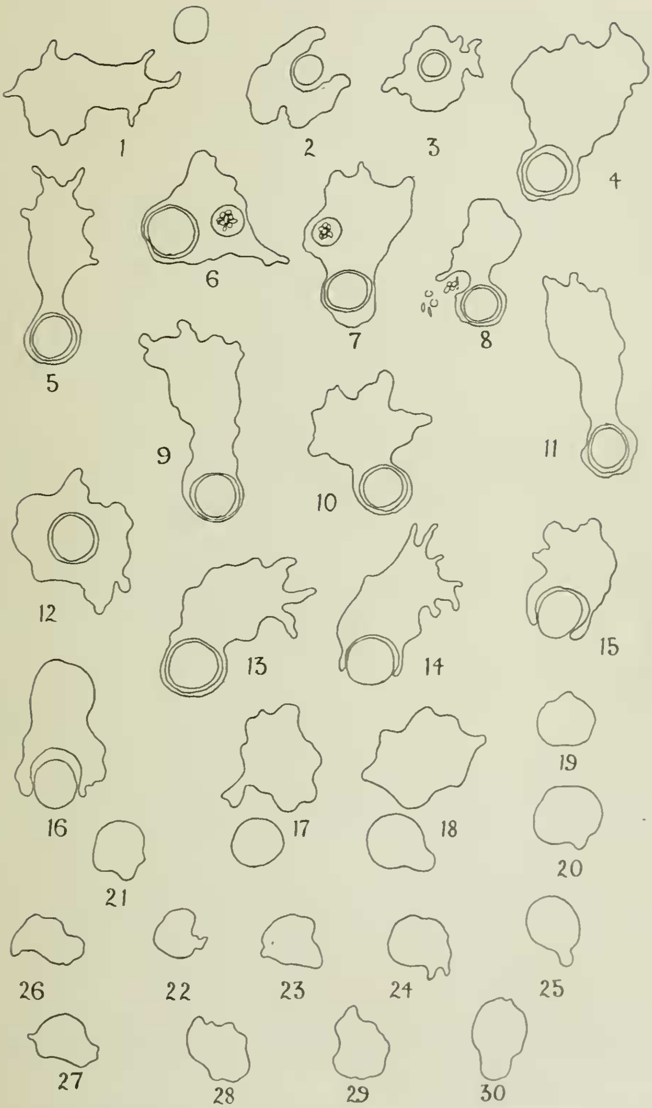
The spheres could be squeezed out of the amoebae by gentle pressure on the cover-glass, and then lay quite motionless and spherical in the water near by. Two such squeezed out on July 21, 1920, at 2.30 p.m., remained quite unchanged until 11 a.m. on the following day. It was also noticed then that numbers of such free, motionless spherical bodies, resembling rounded-off amoebae, could be found in the cultures. Doflein (**9**) states that, in old cultures of Amoeba vespertilio which had become foul and acid in reaction, the amoebae tended to round off and to die. Two questions therefore arose:

(1) Were these rounded bodies in my cultures individuals which had rounded off ? and (2) Were these rounded bodies the same as the spheres which had been seen inside the amoebae, and had the amoebae been extruding them into the culture ?

In order to throw some light on these questions a series of amoebae containing spheres was kept under observation, and the fact was established that the spheres were actually extruded by the amoeba very frequently. Text-fig. A gives the successive stages in the process. It is a composite figure drawn from numbers of sketches made during observations on the living object, and it shows that the extrusion of the spheres resembles ordinary defaecation of the undigested remains of food. It should be noted, however, that the ingested sphere was often carried about in the amoeba for a considerable time, and might often remain for some time in the posterior end of the amoeba, separated from the water only by a very thin layer of ectoplasm, giving the impression that it is about to be extruded. Frequently, however, the protoplasm flowed round it again, and it was taken once more into the central part of the endoplasm. Further, when a vacuole containing a sphere was lying near the surface of the amoeba, and an ordinary food vacuole was lying close against it, the two being separated only by a thin film of protoplasm, the food vacuole might discharge its contents, while the sphere remained unaffected and might be taken again into the depths of the endoplasm (Text-fig. A, 8).

There was, therefore, no external appearance which could be taken as an invariable sign that the ingested body was about to be extruded. A sphere might be carried about thus, on the verge, as it were, of extrusion, for a long time, and might then be taken in again ; or it might be suddenly extruded : or it might, when deep in the endoplasm, rapidly approach the surface and be extruded almost immediately after it had arrived there. In one case the process lasted, from the first rupture of the enclosing membrane to the time when the sphere was quite free, about thirty seconds, from which it will be realized that, when once the extrusion had begun, it proceeded rapidly. Further, although the extrusion usually took place

Text-fig. A.




Freehand sketches of living amoebae to illustrate the ingestion and subsequent extrusion of the sphere (rounded-off amoeba).

at or near the end of the amoeba which was posterior in progression, this was not invariably the case. The sphere might be extruded at the side or at any other point. Probably it is correct to say that, when the amoeba was progressing rapidly in one definite direction, extrusion usually occurred at or near the posterior end; but when the amoeba was putting out pseudopodia in all directions and was not changing its position much, extrusion might then occur at any point of its surface. This is probably true of ordinary defaecation also. When the ingested body was about to be extruded it appeared as in Text-fig. A, 13, and was usually, though not always, surrounded by a well-marked vacuole, pinkish in colour, and separated from the water only by a thin layer of protoplasm. This layer became thinner and thinner, until it was reduced to a mere membrane. Finally it was broken at one point. The ingested sphere then seemed to be forced out, slowly at first and then more rapidly, and at the same time the two halves of the enclosing membrane were withdrawn along its sides, so that the opening to the water was widened (Text-fig. A, 14, 15, and 16). A final effort of expulsion then quite suddenly forced the ingested body out and the cavity which it had occupied rapidly closed.

That an active effort of expulsion occurred is suggested by the fact that the ingested sphere did not merely slide out, but was projected by the force of the expulsion well away from the side of the amoeba. This may have been, however, merely the result of the explosion of the fluid vacuole in which it lay.

The important detail to be noted here is the fact that the vacuole containing the sphere sometimes contained diatoms or the partially digested remains of zoochlorellae, as well as the sphere, and that these were expelled with it and lay with it free in the water. This is a small point which suggests that the sphere had been ingested at the same time as the diatoms, that the vacuole in which it lay was a true food vacuole, and that the sphere was an ingested organism and not a body formed by the amoeba itself.

### 4. Description of the Free Sphere.

One of these recently extruded spheres, observed on July 21, 1920, was being rolled over and over by the movements of Paramoecium bursaria in the culture, and was seen to be perfectly spherical. The cytoplasm was clear, containing fine dark-looking grains together with some larger refractile granules, the nature of which I have been unable to determine. In stained preparations the cytoplasm showed a well-marked meshwork structure and the fine grains referred to above were distinguishable, being distributed over the strands of the meshwork and especially heaped up around the nucleus (cf. the description of the amoeba, p. 672).

In the living sphere the nucleus could not usually be distinguished, but in a few cases I was able to detect it. It is possible that the spheres in which it was visible were dead ones.

As has been noted above, in stained preparations the nucleus of the sphere shows the same structure as that of the amoeba itself. While some of the spheres contained no other structure, others, on the contrary, were full of diatoms and other bodies in food vacuoles (Pl. 28, figs. 2, 3, and 5). Sometimes, when the amoebae contained zoochlorellae the spheres also contained them.

The outline of the spheres was very definite, appearing as a dark line, giving the impression that a definite limiting membrane was present. Examination of stained preparations showed, however, that no such limiting membrane is really there, the effect of a membrane being produced by the arrangement of the meshwork structure of the cytoplasm at the surface, an effect which is commonly seen also in rounded-off amoebae.

In spheres observed under the oil-immersion lens, it was noted that, while immediately after extrusion no contractile vacuoles were present, these appeared a short time after extrusion. In no case have contractile vacuoles been seen in the spheres while they are still in the amoebae. They were never present when the spheres were extruded, but they often appeared soon after extrusion. Since their appearance is at least a sign of vitality, some attention was paid to the time of their appearance, their number, and their rate of pulsation.

A series of observations upon many extruded spheres established the fact that the contractile vacuoles appeared in them at irregular intervals after their extrusion. In one case, for example, a contractile vacuole appeared in the extruded sphere in less than a minute after its extrusion, and one minute after extrusion, two contractile vacuoles were present. In another case, however, no change occurred in the extruded sphere until twenty-two hours had elapsed, when two contractile vacuoles appeared. But in the majority of cases one contractile vacuole had appeared in anything up to twenty minutes after extrusion and two were present about half an hour later. The number thereupon generally increased to four, or in a few cases to six or even eight.

It would be natural to assume that the appearance of several contractile vacuoles in the sphere was an exceptional occurrence, perhaps indicating a pathological condition of the sphere itself or unfavourable physical conditions of the fluid in which it lay. The active amoebae in the same fluid also contained more than one contractile vacuole. Indeed, according to Penard (**21**), *Amoeba vespertilio* often possesses two or three. In my cultures some amoebae were certainly seen with only one and others with several, so that no accurate statement can be made as to what is the normal number. But, if the amoebae in the hanging drop contained more than one, it was not remarkable that the spheres should also develop several, when they were extruded into the same chemical and physical environment.

It was, however, noted that the numbers of contractile vacuoles in any particular sphere might change. In spheres which contained four or more this number was often reduced to two, especially in those spheres which, as we shall see below, developed pseudopodia and moved away. The observations on this point were not, however, sufficiently numerous to bear more than the suggestion that the development of numerous contractile vacuoles in the sphere was a temporary reaction to its sudden change of environment, which disappeared as soon as the organism was able to adjust the physical state of its protoplasm to that of the fluid around it.

It may also be suggested that, if the vacuole in which the sphere had been enclosed were a food vacuole, the sphere, when set free, would be suffering from the effects of the attempt of the amoeba to digest it and would therefore naturally be in a pathological condition, and that the contractile vacuoles would be among the first of the organellae to betray this condition.

The contractile vacuoles arose deep in the protoplasm of the sphere and could be seen to move to the surface, when they were ready to burst. Often in doing so they glided between the granules in the protoplasm, and were then compressed into a dumb-bell shape when they passed between the granules, much as an air bubble is distorted when it is pressed under a cover-glass.

The pulsation rate was not very regular. It varied from as much as one contraction every quarter of a minute to one every six and a quarter minutes, the average being about every minute or rather less. Doflein's experiments (9) showed that high concentration in the medium, such as would be likely to occur in a hanging drop, induces slow pulsation and a decrease in size of the contractile vacuole. Some such influence probably in part explains the irregularity observed here ; but no definite evidence can be offered either in support of or against this view. The slowest pulsation seemed to occur in the spheres with several contractile vacuoles.

When several contractile vacuoles were present they often burst simultaneously, leaving the sphere free from them ; but when only two were present they seemed to alternate, one bursting while the other one grew, so that the sphere always contained one. Further, when several were present, two half-grown ones often fused to form one larger one, which then moved to the surface and burst.

The contractile vacuoles did not appear constantly in any one position in the sphere, but, after bursting, might reappear anywhere. That this is not a false impression produced by rolling over of the sphere is shown by the fact that it was observed in perfectly motionless spheres, and also by the fact that when the bursting of one set of four was delayed the second set of four might appear all in different positions from the first set, so that the sphere appeared to contain more than four contractile vacuoles.

The appearance of odd numbers of contractile vacuoles in this way, their occasional coalescence, their irregular pulsation rate, their multiple number, often subsequently reduced, together with the presence of several contractile vacuoles in the amoebae in the same preparations, suggested that abnormal phenomena were being witnessed. The physical conditions of the hanging drops were probably responsible for some of these irregularities. But the absence of contractile vacuoles from the spheres while they were still in the amoebae, and their appearance in them after they were set free, proved, at any rate, that the spheres were not mere dead defaecated matter, but were alive and were attempting to adapt themselves to the sudden change in their environment.

This view was confirmed by the occurrence in some, though not by any means in all, of the spheres, of tentative amoeboid movements, which, in a few cases, resulted in the sphere being transformed into an active small amoeba.

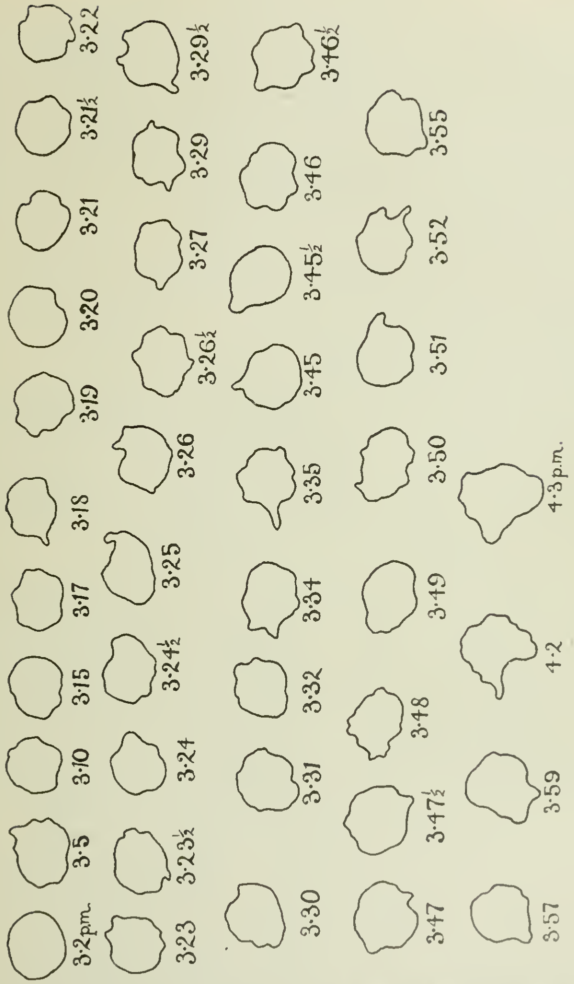
### 5. Amoeboid Movements in the Free Sphere.

In several cases spheres which were extruded under observation were kept under observation for several days, in the hope of some change being observed in them. In most of these cases the only change was the appearance of contractile vacuoles, the pulsation rate of which gradually became slower and slower, until they stopped and the spheres disintegrated.

In other cases, however, the spheres not only acquired contractile vacuoles, but also exhibited slight amoeboid movements. These were often no more than slight changes of form, but definite small pseudopodia were sometimes put out (Text-fig. B). In other rare cases the sphere became transformed into a small active amoeba, which moved out of the field of observation.

Text-fig. B represents drawings made with the camera lucida of the changes undergone by such a sphere, and in Text-fig. A are freehand drawings of another case. It is interesting to note that, although in the period between extrusion and the

TEXT-FIG. B.




Outlines of an extruded sphere (rounded-off amoeba), drawn with the camera lucida at the intervals of time stated in the figure, to show the amoeboid movements often performed by the sphere after it had been extruded.

appearance of the pseudopodia the number of contractile vacuoles might vary from one to eight, it had always been reduced to two at the most, by the time that the amoeboid activity of the sphere had been well established.

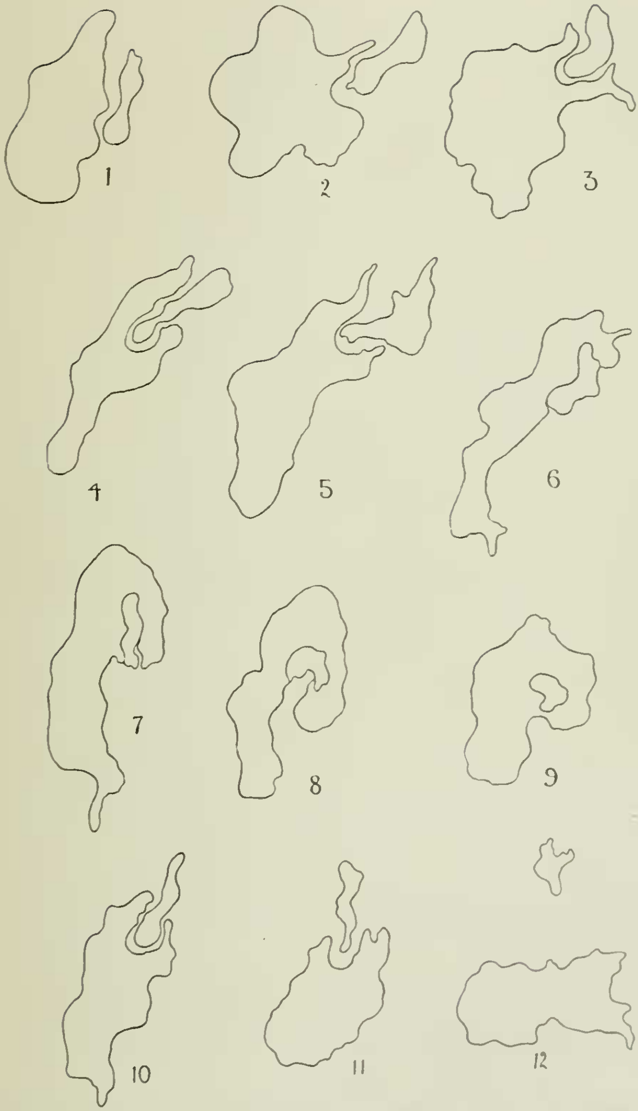
## 6. Ingestion of the Sphere.

The extrusion of the spheres and the development of some of them into small amoebae had been seen before I was fortunate enough to observe an amoeba actually ingesting a free sphere. I had just watched the extrusion of this sphere, and the amoeba which had extruded it had hardly moved out of the field before another amoeba entered and immediately took up the sphere which the other had left behind. Further observation showed that this fate was suffered not only by the motionless spheres but also by those which had already become transformed into small active amoebae.

The process of ingestion was perfectly normal in every way. The big amoebae put out pseudopodia round the sphere and gradually enclosed it in a typical food vacuole. The result was an amoeba containing a sphere, exactly resembling the original amoeba, with a sphere inside it, which had awakened my interest at the beginning of the observations.

Not long afterwards I was able to follow and to sketch the dramatic chase of a small Amoeba limax by a large active Amoeba vespertilio. Text-fig. c gives the details of this drama. It will be seen that the large amoeba at first attempted to surround its prey (4, 5, and 6), and, after cutting off its retreat, nearly succeeded in enclosing it (7 and 8). At 9 the small amoeba is not inside the large one, but underneath it, the Amoeba vespertilio having streamed over the Amoeba limax so as to hold it between itself and the glass. I have often seen Amoeba proteus capture Paramoecium and other Ciliates in the same manner. The Amoeba limax, however, was too nimble in this instance, for it escaped again (10) and the large amoeba made no further attempt to capture it. A similar case has been described and figured by Jennings (**16**), in which the amoeba also failed to secure its prey. Jennings concluded that the behaviour of the captor to the victim could not be explained as the result of chemical or tactile stimuli only, but that there was a finely co-ordinated adaptation of the movements of the captor to those of the victim,

TEXT-FIG C.




Freehand sketches of the chase of an amoeba of the 'limax' type by an *Amoeba vespertilio*, and the partial ingestion and subsequent escape of the former.

a conclusion with which I am entirely in agreement. The behaviour of Amoeba proteus in its capture of large Ciliata like Paramoecium and Colpidium in cultures strikingly supports the same view (cf. also Schaeffer, **23**).

Penard (**21**, p. 700) has described another instance of the chasing of one amoeba by another which ended in the fusion of the two, and Leidy (**18**) has described and figured what is undoubtedly the successful capture and digestion of an Amoeba verrucosa by Amoeba proteus. This latter case is particularly interesting, since Leidy says that the A. verrucosa assumed, in the body of its captor, 'the appearance of a large sphere, still retaining its contractile vacuole unchanged'. Later on the 'victim had become pyriform and striate, and was then included in a large water vacuole. Still later the body of the A. verrucosa appeared to have become broken up into five spherical, granular balls. . . .' Leidy was unable to follow the ultimate fate of these 'granular balls', but he supposed that they were digested. A comparison of Leidy's figures with those illustrating this paper leaves no doubt that he was dealing with an isolated instance of a process which was occurring on a larger scale in my cultures.

The fact, however, that Leidy's is the only one of these cases in which anything resembling actual digestion was seen, and the fact that I have only in one instance (cf. below) seen in my cultures doubtful evidence of digestion of the spheres, suggest that the amoebae only rarely are able to digest other amoebae which they may capture. Further, it seems probable that amoebae only rarely even attempt to capture other amoebae, and usually fail to retain these when they are active, however frequently they may succeed in ingesting them when they are sluggish or resting in a rounded-off condition.

The case in which the doubtful evidence, referred to above, of digestion of a sphere was seen, was that of a sphere which was spherical when it was ingested, but which did not remain so. It underwent distinct form changes while it was still inside its captor. Pl. 29, figs. 8 and 10, represent other ingested spheres, drawn from stained preparations, which had assumed an irregular form while inside their captors. In the case just referred to, which was kept under observation, the sphere returned to the spherical form after it had been inside the amoeba about five hours. Later it became less and less distinct, and seven hours after it had been ingested it could no longer be distinguished. Apparently it had been digested. This was the only instance in which anything like digestion of the spheres was seen. In all other cases which were kept under observation the spheres were sooner or later extruded by the amoebae which had digested them.

One other point remains to be mentioned before we discuss the nature of the spheres. It is illustrated by Pl. 28, figs. 1, 2, and 4, and Pl. 29, fig. 7, drawn from stained preparations, in which several examples of it were found at different dates. Pl. 28, figs. 1 and 2, show the phenomenon in its most typical form, and it will be seen, on reference to them, that there are here as many as four amoebae, enclosing one another, giving the impression of concentric fission. The figure looks, at first sight, like the dream of a pre-formationist, but we shall see that it has a much more prosaic explanation. It is so remarkable that I at first believed it to be an artefact, due, I supposed, to drying of the preparation, or to imperfect fixation. The other organisms on the slide were, however, well fixed and stained, and these remarkable structures did not occur on slides of one batch only but were present on slides made on widely different dates. Further, I saw what I interpreted as the same structures in the living organisms, although I was never able to convince myself of this. In any case the phenomenon admits, as we shall see, of a perfectly natural explanation if we adopt the only hypothesis which fits the whole of the facts.

There can be no doubt that there are actually several independent amoebae enclosing one another, because their nuclei are perfectly distinct and each amoeba possesses a vacuole for the reception of the others. The nuclei are, moreover, all exactly similar in structure to one another. Pl. 29, fig. 7, is perhaps the most remarkable and was the most difficult to interpret. There are here present seven nuclei, and the interpretation of the figure is best deferred to a later stage (cf. below, p. 700).

## 7. Discussion.

Three main possibilities suggested themselves as explanations of the observations just described.

First, the spheres may have been parasites; secondly, they may have represented some form of reproduction, such as endogenous budding; thirdly, they may indeed have been food bodies, the amoebae having ingested other amoebae of the same or other species. On this last view, the phenomena were those of 'cannibalism'. As the title of the paper shows, I believe this last to be the correct interpretation.

In order to give my reasons for this conclusion, it will be necessary to discuss these three views in turn.

### (1) The Parasite Hypothesis.

At first this view seemed very probable. The spheres resembled, at first sight, organisms like the Suctorian Sphaerophrya, which is so common a parasite of Ciliata in cultures. Closer examination of them quickly proved, however, that not only did the spheres never show any structure resembling tentacles but also that no Suctoria were ever present in the cultures. Further, the nucleus of Sphaerophrya is not vesicular. The spheres, in fact, did not show any single feature by which they could be classified as Suctoria.

Prandtl (**22**) has described a Thecamoebidan, Allogromia, which became parasitic upon Amoeba proteus, Arcella, Nuclearia, and Paramoecium in order to accomplish its sexual cycle in their interior. This organism, however, does not in any way resemble the spheres described above. Not only were no shelled Rhizopods ever seen in any of my cultures, but the structure of Allogromia, its possession of chromidia and the changes which it undergoes in its host, together with the fact that it is capable of reducing its host's vitality, definitely exclude any possibility that the spheres were parasites of this nature.

Buck (**1**) has described, under the name Phonergates vorax, another shelled Rhizopod, identical, according to

Bütschli (2), with Lecythium hyalinum, which also may become parasitic upon Amoeba proteus, Rotifera, Crustacea, &c., during its sexual cycle. Buck states that this organism may, when it is parasitic in Amoeba and other organisms, closely resemble Sphaerophrya. But I am convinced, after reading his paper and studying his figures, that Phonergates has no points of resemblance to the spheres here described, except, perhaps, that it is about the same size.

Penard (**21** *d*) has described in an amoeba which he names Amoeba alba, a parasite similar to one seen by Buck in Arcella and later found by Dangeard (7*c*) in the Heliozoa Nuclearia simplex and Heterophrys dispersa and called by him Sphaerita endogena. The form seen by Penard belongs to the Chytridiaceae, and he thinks that it is similar to that described by Chatton and Brodsky (**5**) in Amoeba limax. The latter authors discuss the whole question of these and allied parasites, and it is obvious that none of these parasites resembles the spheres described above.

Another parasite, Nucleophaga amoeboea, allied to the above, has also been described by Dangeard (7*b*), Penard (**21** *d*), and others. It attacks the nucleus of various amoebae. Doflein (**9**) has further described the formation of giant nuclei in Amoeba vespertilio, which is the amoeba with which we are dealing, due to a parasite which he regards as being closely allied to, if not identical with, the Nucleophaga of Dangeard. The spheres described above have, however, obviously nothing to do with this or any other nuclear parasites, since the nucleus of the amoeba containing the sphere was always intact and normal and the sphere itself had a nucleus of its own, which was very similar to that of the amoeba which contained it.

Leidy (**18**) has described and figured a number of interesting inclusions in Amoeba proteus and other species. His observations were, however, made upon the living object only, and it is unfortunately impossible to determine from his figures and descriptions what was the real nature of these inclusions. Some of his figures of them, described by him as nuclei, might equally well be interpreted as parasites of the Chytridiacean type referred to above. On Pl. viii, figs. 12–16, of his book he figures a 'multinucleate' Amoeba villosa, and in fig. 15 he shows a process which he describes as the bursting of the nucleus and the expulsion of its coarsely-granular contents. He was almost certainly dealing here with a Chytridiacean parasite and not with a multinucleate amoeba at all. Doubt must, therefore, be entertained as to whether his other figures of the nuclei of the various amoebae described by him really represent the nucleus. It is doubtful, for example, in the case of the form of 'Amoeba proteus' which he figures in Pl. viii, figs. 17–28, and describes on p. 53; and also of those shown in Pl. iv, fig. 25, also of 'Amoeba proteus'. The same doubt applies to the nucleus of Dinamoeba (Pl. vii, figs. 5, 7, and 8) described on p. 91 as a 'large, pale granular nucleus, surrounded by a clear halo', an appearance which the true nucleus of Amoeba proteus rarely or never presents. It is much more likely that what he saw was either a parasite or some other granular organism which had been ingested. The excellence of Leidy's observations in general leads one, however, to accept most of his interpretations, and it is to be remembered that, without the control of stained preparations, mistakes of this kind are almost unavoidable.

Wallich (**29**) records a number of observations upon living Amoeba villosa, but in this case also it is practically impossible, in the absence of stained preparations, to determine exactly what he was dealing with. In the first place it is doubtful whether the bodies which he regarded as nuclei were in reality nuclei at all. If they were, it is probable that they were, as some of Leidy's undoubtedly were, nuclei infected with a Nucleophaga. And Carter (**4**, **4** *a*) probably fell into the same error.

It became obvious, therefore, that the spheres showed no resemblance to any of the parasites of amoeba of which a full description was available. The following general considerations also contributed to the abandonment of the parasite hypothesis.

First, the sphere did no damage to the amoeba which contained it. At any rate no damage was demonstrable, and the amoebae lived and multiplied normally while they contained spheres, and are, indeed, still living at the present time in the same cultures, although only rarely do they now contain spheres.

Secondly, if the spheres were parasites it is difficult to understand why they were so frequently extruded by the amoebae. When a parasite has gained entrance to its host it usually does not leave it, except for the purpose either of propagative reproduction or of mechanical distribution of its species. Such a parasite would, at some time or other during its sojourn in its host, be likely to show some evidence of its reproductive cycle. The spheres, however, never showed any signs of any reproductive capacity whatever, either when inside or outside the amoebae. They were taken in and passed out in the same manner as ordinary food matter would be ingested and extruded, behaving in a strictly passive manner.

It occurred to me that the amoebae and the spheres might be symbionts or commensals. Against this highly improbable theory was the fact that a vacuole, filled with fluid, was present round the sphere. In other cases of symbiosis among the Protozoa, as, for example, that of the zooxanthellae and zoochlorellae, the latter occurring under certain conditions in the very amoebae under consideration, no vacuole surrounds the algae.

### (2) The Hypothesis of Endogenous Budding.

The second hypothesis, that the spheres were endogenous buds, was much more attractive and led me astray for some time. I should have liked to have been able to prove that they were buds, and very nearly succeeded in convincing myself that they were. But the finding of such structures as those shown in Pl. 28, figs. 1 and 2, and Pl. 29, fig. 7, where two, three, or four amoebae were enclosed within one another, seemed to stretch the theory of endogenous budding rather far. Before ascribing such remarkable structures as these to endogenous budding it seemed wise to reconsider the data. When this was done it became obvious that the spheres were not endogenous buds.

Throughout my stained preparations I have never seen any signs of change in the nucleus, either in the amoeba or in the sphere, although I have very carefully searched for such cytological evidence of the formation of a bud. Whatever the size of the amoebae or of the spheres might be, the nuclei of both were always in the same condition, that is to say, in the 'resting' condition which has been figured; the nucleus of the sphere was always similar in structure to that of the amoeba.

I have tried hard to find evidence of the division of the nucleus of the amoeba to form the nucleus of the sphere, or evidence of the formation of the latter from chromidia extruded by the nucleus of the amoeba. Indeed, under the influence of the hypothesis of endogenous budding I have often thought I have seen chromidia, just as I have often thought I have seen in this and in other forms, centrosomes, centrioles, and other structures, when I have wanted to find them. But these structures have, on re-examination, proved to be, in every case, either figments of my own imagination based upon improperly differentiated slides, or artefacts. I am now convinced that there is no evidence, of any sort or kind, of changes in the nuclei either of the amoebae or of the spheres in my slides.

If endogenous budding had been going on to the extent that the abundance of the spheres would suggest, some evidence of the mode of formation of their nuclei from the nuclei of the parent amoebae would have been seen. It is true that even binary fission is seen only very rarely, as Doflein also points out (**9**). In my slides I have seen only two or three dividing amoebae, and in those the two daughter nuclei had already returned to the 'resting' condition. This is the only evidence that I have seen, either in the stained or in the living material of any reproductive processes whatever.

It is to be remembered, moreover, that when the endogenous buds are being formed in an organism like the Suctorian Dendrocometes paradoxus, the contractile vacuole is present while the bud is still within the parent. It is, in fact, one of the first of the organellae to appear, and its presence can be taken as an indication that bud formation is in progress (Lapage and Wadsworth, **17**). In the spheres, on the contrary, a contractile vacuole was never seen while the sphere was within the amoeba. It did not appear in the sphere until an appreciable interval had elapsed after the sphere had been expelled.

Further than this, endogenous buds, in other groups of Protozoa, do not usually vary much in size in any particular species producing them. They are cut out of the parent to a definite size which remains unaltered, and it is not true that they are smaller when they are first formed and that they grow to a mature size before their birth. The spheres, however, although they show a striking uniformity of structure, do vary a good deal in size, some being as small as 10 $\mu$ in diameter, others up to 46 $\mu$. This variation in size suggests that they are not endogenous buds. Further, in the smallest ones the nucleus is fully formed and typical, measuring 6 $\mu$ in diameter, the endosome measuring 3 $\mu$ in diameter. This is a significant fact, when we remember that the nucleus of the A. limax also present in the culture is 5–6 $\mu$ in diameter with an endosome of 3 $\mu$. The variation in size of the spheres is, therefore, more simply explained on the hypothesis that they represent amoebae of different sizes which have been ingested, than in any other way.

The fact that some of the spheres developed, after they were extruded, into typical small amoebae certainly suggested that they were reproductive bodies; but this was just as easily explained as the escape of an ingested amoeba after successful resistance to the digestive juices of its captor, and such an explanation was more in accordance with the other facts.

Another important fact against the view that the spheres were endogenous buds was the observation that the spheres, while inside the amoebae, often contained diatoms and other food matter in food vacuoles (Pl. 28, figs. 2 and 5, and Pl. 29, figs. 7, 8, and 9). This is highly significant in view of the fact that the amoebae in the culture were all feeding principally upon diatoms. Endogenous buds, when they are formed in other groups of Protozoa, are invariably free from food vacuoles until after they are born, and it is indeed difficult to imagine how they could obtain any solid food until they are set free. Even if we adopted the fantastic view that, in the case under consideration, the spheres had obtained the diatoms from the amoebae in which they lay, it is impossible to explain how they did so, seeing that a vacuole filled with fluid lay between them and the protoplasm of the amoebae. The presence of that vacuole is, of course, in itself no argument against their being endogenous buds, since most endogenous buds develop inside a cavity or ' brood chamber ' in the parent.

A still more significant detail is, however, the observation, made upon the living object, that, when the sphere was extruded, the remains of diatoms might be extruded with it from the same vacuole. This can only mean that the diatoms were taken up at the same time as the sphere, a fact which is easy to understand when we remember that the amoebae were feeding mostly in the clumps of diatoms and débris in the culture rather than in the open. The vacuoles in which the spheres lay were, therefore, true food vacuoles and not of the nature of ' brood chambers '. This does not prove, of course, that they were not buds, since the amoebae were seen to ingest the free spheres, and it might be argued that the spheres were no less true buds because their parents were eating them. But, taken in conjunction with the absence of any evidence of the mode of formation of buds and the presence of food vacuoles in their cytoplasm, it is a very significant piece of evidence.

Another observation pointing in the same direction is the fact that the spheres were not always perfectly spherical, but were often irregular in shape and, indeed, were, in some cases, seen to undergo form changes while inside the amoebae (cf. p. 686, supra). This strikingly suggests that they were amoebae which had been ingested.

The hypothesis of endogenous budding breaks, however, on the same rock as did the parasite hypothesis. It fails to explain the occurrence of several amoebae enclosing one another, as are shown in Pl. 28, figs. 1 and 2. This could, it is true, be interpreted as endogenous budding with pathological delay of the birth of each bud, so that an appearance of concentric fission resulted; but there seems to be no necessity for so fantastic a view, when the structure can be explained naturally and simply as the result of cannibalism.

Lastly, it is difficult to understand why endogenous budding, if it occurs in the Amoebaea, has not been fully described already, seeing that such a vast amount of work has been done on these organisms. It is true that Penard (21) has made several references to the occurrence of so-called ' embryos ' in Pelomyxa and in various amoebae. With regard to Pelomyxa, he says that ' in the month of October, 1900, the greater part of the individuals examined contained, in their bodies, true embryos. These embryos, apparently swimming in the plasma, . . . showed as little grey masses, spherical, ovoid or pyriform, in the interior of which one saw some little, brilliant grains, one or two vacuoles and a vague appearance of nuclei. Isolated by compression of the Pelomyxa the embryos pushed out slowly prolongations in the form of little waves or lobes and continually deformed themselves in their entirety.' He was also able to convince himself of the presence of a contractile vacuole, which ' only functioned in a lazy manner ', and he was sure of the presence of a ' nucleus, round, with a nuclear membrane already formed and distinct, with nuclear sap and a central nucleolus and one or two other spherules. . . . which seemed to represent nuclei also '. He adds his opinion that ' the presence of these embryos, living in good health in the plasma of the Pelomyxa and usually multinucleate, seems to me to indicate that they are products of the animal itself and not parasites '.

This description suggests that he may have been dealing with either parasites or with amoebae of the Amoeba limax type which had been ingested by the Pelomyxa; but doubt is thrown over the whole of the observations by his statement, on another page of the same work, that he believes with Greef that the so-called 'Glanzkörper' of Pelomyxa develop into small amoebae similar to those which he saw pass out of the Pelomyxa. From his account it seems likely that he has confused various different structures, true 'Glanzkörper', fungal and Flagellate food, and ingested small amoebae. This is only another instance of the difficulties which arise, especially for other workers, when observations on living specimens are not controlled by properly made permanent preparations.

Penard, in the same work, makes other references to the occurrence of similar 'embryos' in the amoebae which he names A. nitida, A. villosa, A. annulata, A. nobilis, A. terricola; and in Rhizopods like Difflugia, Diaphorodon, and, above all, in Nebelidae, he found bodies which he thought may have been reproductive in nature. In most of these cases he gives figures which certainly suggest strongly that he was dealing with amoebae which were ingesting and extruding again other amoebae of the same or other species. In the 'embryos' of A. nobilis he saw 'little diatoms' and 'little grains which appear to proceed from digestion'; and those of A. nitida contained 'the appearance of little grains of starch or little diatoms, which themselves seemed to be in course of digestion'. But he does not seem to have thought it necessary to explain how these 'embryos', while inside their 'parents', had been able to ingest their diatoms. It seems very likely that these 'embryos' were similar in nature to the spheres in my amoebae and that Penard fell into the same error as that from which I was only saved by the study of permanent preparations.

Grosse-Allermann (**13**), in a study of Amoeba terricola, saw, in two instances only, a swollen amoeba full of small spheres of 30–40 $\mu$ in diameter, and he supposed that he was dealing with the end result of multiple fission. Penard (**21** *d*) saw somewhat similar phenomena in the same amoeba, but regarded the spheres as parasites which had developed inside the Amoeba terricola and which were set free by its death.

Much more plausible, however, are the accounts of endogenous budding in amoebae given by Liston and Martin (**19**), Wherry (**30**), and Hogue (**15**). The last-named worker also describes the formation of ' exogenous ' buds, by the streaming out of chromatin granules from the karyosome into the ectoplasm, where they collect to form the nuclei of the exogenous buds. Her figures and description, however, suggest that the so-called chromatin granules were either artefacts or parasites like the Chytridiaceae referred to above.

Hogue's figures of the endogenous buds, like those of Wherry, are much more convincing and show a striking resemblance to the figures illustrating this paper. Neither of these workers, however, has given a detailed description of the so-called ' buds ', nor was the development of the ' buds ' followed. Had this been done in all probability a different conclusion as to their real nature would have been reached. It should be noted, also, that in both these cases the amoebae were studied in agar media, which cannot be regarded as a sound method of cultivating these organisms. Further, the cultures were crowded with amoebae, a state of affairs which would tend to encourage the ingestion of the amoebae by one another.

Liston and Martin (**19**) have described endogenous budding in a large amoeba from liver-abscess pus. This amoeba also was studied on agar media. Liston says that he saw an amoeba develop three or four ' buds ' within its body while under observation and that these were liberated. Older and larger amoebae might contain as many as six ' buds ' in various stages of development. If this were so, it is unlikely that they were true endogenous buds at all, because endogenous buds are usually formed of a certain definite size which does not increase or change before they are born. Liston also states that the ' buds ' became recognizable in the amoebae ' when a larger mass of chromatic material was assembled than could be reasonably explained on the supposition that it was formed from ingested bacteria ', that the ' buds ' were formed around these masses of chromatic material, and that these masses then became the nuclei of the ' buds '. Martin, in a study of the stained material, confirms this and says that the nucleus of the 'bud' is formed from 'chromidia contained in it when it is first formed and derived from the chromidia scattered through the cytoplasm of the parent'.

He also says, however, that 'the nucleus of the amoeba takes no direct part in the formation of the bud. There is absolutely no evidence, either from observation on the live amoebae or from the stained films, for any form of nuclear division connected with the bud formation.'

This latter statement might equally well have been made about the spheres described in this paper. When it is remembered that I also, under the influence of the view that the spheres were endogenous buds, found in my amoebae structures which could easily be interpreted as chromidia, the parallel is complete.

Upon re-examination of my preparations, however, I have been unable to convince myself that the fine grains in my amoebae were chromidia at all, and certainly I have never seen anything resembling a collecting together of these grains inside the spheres to form their nuclei. All the spheres had a fully-formed vesicular nucleus. While I must admit, therefore, that Martin may have been dealing with something quite different from my spheres, I still am of the opinion, without desiring to impugn his high reputation as an accurate observer, that his 'buds' were in reality of the same nature as my spheres, that is to say, that they were amoebae of the same or another species which had been ingested.[1] Two types of amoebae were present in the cultures of Liston and Martin, and it is possible that one kind was ingesting the other. The method of cultivation of these organisms upon agar

[1] Dr. H. M. Woodcock, of the Lister Institute, first suggested to me, in 1920, that the 'buds' described by Liston and Martin were probably not true buds at all and thus gave me the clue to the real nature of the spheres in my own cultures. Recently Dobell and O'Connor (8*a*) have expressed the same opinion. Compare, also, the still more recent remarks of Woodcock (32) with regard to the need for care in the interpretation of cultural forms of Protozoa.

media might be expected to induce them to exhibit abnormal behaviour in this and in other respects.

It is much more probable that Wallich (**29**) also saw something similar to the observations recorded in this paper, since he figures a small amoeba which he calls a 'gemmule' and believed he had proved the occurrence of 'gemmation' and 'viviparous reproduction' in Amoeba villosa. His 'viviparous reproduction' seems to rest upon the occurrence of many small amoebae in his cultures, such as also occurred in my own, and it is probable that his 'gemmule' was either an amoeba which had become rounded off or one which had been recently extruded, after having been ingested. He also describes structures which he calls 'nucleated corpuscles' and 'sarcoblasts', and he says that the 'sarcoblasts' are obviously reproductive, because, although he never saw them develop into amoebae while they were yet within an 'amoeba cyst' (a structure which is obviously not a cyst, but a dying amoeba), yet he saw bodies present in the same fluid at the same time, outside and identical in appearance, which did develop into amoebae! Since he made no permanent preparations, it is not possible to know what he really was dealing with, but it is unlikely that either the 'sarcoblasts' or the 'nucleated corpuscles' were in any way similar to my spheres. Wallich, however, further describes what he refers to as 'a process resembling gemmation or viviparous reproduction'. His figure of a 'gemmule' is very like the recently extruded sphere of my cultures, but since Wallich says that he never saw his 'gemmule' emerge, and further that he is 'unable to vouch for' the process of gemmation 'on his own authority', it is not possible to attach much importance to his observations.

While there are, therefore, several references to the occurrence of endogenous budding in the Amoebaea, there seems to be no single record of it which is free from doubt and certainly no record which has been confirmed by subsequent workers. This is a curious fact, when we remember that endogenous budding does occur in forms so closely allied to the Amoebaea as Arcella and other Thecamoebida. It even suggests that, either some of the cases cited above are correctly interpreted as instances of endogenous budding, or that, alternatively, the Thecamoebidae are not so closely allied to the Amoebae as has been thought.

All these considerations shook my belief in the very attractive view that I was witnessing an epidemic of endogenous budding.

### (3) Hypothesis of Cannibalism.

Turning to the third alternative I found that the cannibalism hypothesis not only explained those facts which the other views explained, but explained them much more simply and readily. In addition, it did not fail where the other two views had failed. This hypothesis provides the simplest explanation and it covers all the facts without introducing into the already complicated problem of the life-history of amoeba a new and hitherto unauthenticated process.

Further, it explains simply enough how such structures as those shown in Pl. 28, figs. 1, 2, and 4, and Pl. 29, fig. 7, can arise. These structures are explained in detail in the text explaining the figures. It is sufficient here to say that such structures arise by the ingestion by amoebae of other amoebae which had previously themselves ingested yet other amoebae, a process which can give rise to the most remarkable and complicated structures. Such phenomena must be pathological. Whether cannibalism itself is pathological is a matter of opinion, in the present state of our knowledge. That it is not a frequent occurrence is shown by the paucity of references to it in the literature, although Doflein (**9** *a*) says that he has often seen cannibalism, i.e. the eating by amoebae of young forms or of cysts of their own species, and that such occurrences have given rise to statements about internal budding and formation of embryos.

An amoeba, in the absence of its normal diet, will eat almost anything. In my own cultures of Amoeba proteus, for example, these organisms, which were thriving upon a diet of bacteria, became voracious carnivores when they were supplied with *Colpidium colpoda*; and Doflein has recorded a similar fact (**9** *b*). It is not surprising, therefore, that an amoeba like *Amoeba vespertilio*, which feeds normally upon diatoms and had been kept for many years in an old hay infusion in which its normal food supply must have been for long scarce, and in which Paramoecium and other Ciliates were present, should have turned, under the stimulus of the change of environment provided by the sub-cultures, to the ingesting, not only of the diatoms which developed in those sub-cultures, but also of other amoebae, both of its own and of other species.

At first I was inclined to think that starvation played a part in causing the amoebae to become cannibalistic. They showed, however, few other signs of starvation. They exhibited normal activity, they multiplied abundantly, and, beyond what was probably a more marked vacuolation than is usual for the species, were in no other way abnormal. They are still living in the same dishes, although they have been practically untouched for two years; but they only occasionally now ingest one another, and are feeding actively upon algae which have developed in the cultures.

It is, moreover, by no means certain that in 1920 they were ingesting their own species alone. Though this probably occurred often, in other cases a comparison of the sizes of the spheres and especially of their nuclei with those of the other amoebae present in the cultures (cf. supra, p. 675) suggested that the small spheres were mostly ingested examples of *Amoeba limax*. Many of the medium-sized spheres might equally well have been either large individuals of *A. limax* or small examples of *A. vespertilio*.

In this connexion the interesting question arises as to whether an amoeba, even if it ingest a member of its own species, can digest it. I have only been able to follow, in the living object, one case of what appeared to be the digestion of the ingested sphere (v. also supra, p. 686). In the stained preparations spheres were often seen, of all sizes, which took the stain more feebly than the others on the same slides, the nucleus often not staining at all. These may have been spheres which were undergoing digestion, or they may have been merely dead ones. In the majority of cases the spheres certainly seemed to resist digestion, although it was evident that most of them were killed by their sojourn in the food vacuole or were, at any rate, so much damaged that they were unable to resume their activity after they were extruded. The appearance of a contractile vacuole in them indicated an attempt at the resumption of vitality ; but usually the attempt went no further and the extruded spheres disintegrated if they were not again ingested. In a few cases abortive attempts at amoeboid movements occurred ; and in fewer still these were successful and the sphere became transformed again into a small amoeba which was apparently little the worse for its experience.

It is evident, therefore, that the amoebae found difficulty, at least, in digesting other amoebae which they took up. They might, therefore, extrude them again, just as they will extrude other indigestible material. If these extruded amoebae had been killed by their sojourn in the food vacuole or died soon after extrusion they might be again ingested by other amoebae ; and it is probable, although I can produce no evidence to prove it, that these dead or dying amoebae could be digested. One is reminded here of the fact that, in Vertebrates, the gastric juice does not digest the mucous membrane of the stomach, unless that is damaged or in a pathological condition, but that post-mortem digestion of the stomach can and does occur.

Another reason for the extrusion of the spheres is suggested by the observation of Rhumbler, as quoted by Minchin (**19** *a*), that amoebae disgorge any food matter that they may contain under the influence of strong light, such as that to which they are subjected when they are brought into the field of the microscope. That this is not the only reason in this case is shown by the frequent occurrence of free spheres in the cultures themselves, before any of the fluid had been examined under the microscope. They could be picked up from the bottom of the culture dishes with a pipette, and must, therefore, have been extruded in the cultures where the stimulus of strong light did not operate. Drying of the slide might conceivably have caused extrusion, as Wallich also suggested (**29**). But this factor also would not operate either in the cultures or in the preparations used for observing living specimens.

To return to the question of what caused the amoebae to become cannibalistic, I am unable to offer any intelligent suggestion. It has already been mentioned that the cultures were not unhealthy, since the amoebae throve and multiplied, as did also the Ciliates and other small amoebae. The balance of evidence showed that the amoebae were not to be regarded as starved, and certainly not as so starved that they resorted to utilizing their own kind as food, a condition which must be rare in both natural and artificial conditions. Further, we have seen that it is at least very doubtful that they were really feeding at all on the amoebae which they ingested, since the evidence is that they could only very occasionally digest them. Their condition seems to have been like that of the army recruit, who, when he asked for a drink on the march, was told to suck a stone.

A possible explanation may be sought in the view that the amoebae had become so numerous in the cultures that the active ones were ingesting the rounded ones and, finding them indigestible, were extruding them again. Schaeffer's work on the feeding habits of amoebae (**23**) is interesting in this connexion. He found that the ingestion of particles by amoebae is not to be explained entirely by chemotaxis, but that other factors operate, especially movement, either natural or mechanical, in the material offered, the nature of the amoeba itself, i. e. whether it were 'raptorial' or not, the physical similarity to or difference from the normal diet of the material offered and the degree of hunger from which the amoebae were suffering. He found, for example, in his experiments with carmine grains, that the amoebae got rid of these much more quickly than normal food matter, and generally as soon as possible. Also he thought that the carmine was extruded because it was actually disagreeable to the endoplasm, though not to the ectoplasm, and not merely because it was indigestible. Further, a piece of carmine was eaten only once if the amoeba was only mildly hungry : several times if it was very hungry ; but the amoebae showed less and less inclination to ingest the same grain if it were offered to them several times in succession. The same was true if a number of different grains were offered, each only once.

It is obvious, therefore, that the factors which govern the feeding of amoebae are by no means simple. It is probably for this reason that I have been unable to induce my amoebae to repeat their performance of 1920, either in the old or in fresh cultures, on anything like the same scale. I have also looked carefully for similar phenomena in thick cultures of Amoeba proteus obtained by the methods of Taylor (**27**) and Doflein (**9***b*). But, although these amoebae often exist in such numbers that they are in close contact, and are actively feeding upon Colpidium and Chilomonas, i. e. upon a carnivorous diet, they have never showed the slightest tendency to ingest one another. Schaeffer (**23**) also found that his amoebae, although they were eating Ciliates and Flagellates readily, never ingested one another. Further, Doflein (**9**), in his study of Amoeba vespertilio, does not mention any case of their ingesting one another. He used, however, chiefly amoebae containing zoochlorellae, whose metabolism must have been, therefore, abundantly provided for even in the absence of their normal diet ; and in my own cultures of Amoebae vespertilio containing zoochlorellae, relatively very few of the amoebae contained spheres, and in those which did the spheres also contained zoochlorellae.

It is very likely, therefore, that the epidemic of cannibalism which is described in this paper was an isolated occurrence, dependent for its causation upon the physical and chemical constitution of the culture medium and also, as Schaeffer's work shows, upon the physiological condition of the amoebae themselves. The fact that, in those other cases in which similar phenomena have been observed in other than isolated individuals and which have been erroneously interpreted as cases of endogenous budding, the amoebae were studied under conditions of artificial cultivation which at least differed widely from the normal environment of the amoebae, is additional evidence in support of this view. Until the methods of cultivating Protozoa are standardized upon the basis of a scientific physical and chemical analysis of the normal environment of these highly sensitive organisms, we must expect that atypical and bizarre phenomena will be witnessed in cultures, and that these will not only be rashly interpreted by the inexperienced, but will also readily mislead even the most careful and conscientious workers.

Reviewing the whole of the facts, I conclude that the hypothesis of cannibalism explains the facts described above readily and simply. It explains the variation in size of the spheres and the similarity of their structure to that of the amoebae which contained them. It explains also their inability to live after extrusion, the presence of food in them while they were still inside the amoebae, and the complete absence of any cytological evidence of the formation of endogenous buds. It affords also an explanation of the ingestion and extrusion and, in some cases, of the re-ingestion of the spheres, and of the remarkable occasional occurrence of several amoebae enclosing one another. I am, however, unfortunately unable to throw any light upon the interesting question as to whether an amoeba can digest individuals of its own species, or to determine what the actual stimulus was which led these amoebae to adopt temporarily the cannibalistic habit.

In conclusion, I am pleased to have the opportunity of recording here my indebtedness to Professor S. J. Hickson, F.R.S., in whose department the work was done, for his kindly interest and help, and to Miss Ann Bishop, B.Sc., and Mr. J. T. Wadsworth, for many very useful suggestions and helpful criticisms.

## Summary.

1. This paper describes the temporary adoption by *Amoeba vespertilio* of cannibalistic habits. The amoebae fre-

quently ingested, but in most cases failed to digest, other individuals of their own and also of other species (A. limax).

2. In some cases, an amoeba, which had ingested another, might itself then be ingested by a third amoeba; and these three might then be taken up by a fourth amoeba, so that remarkable figures, suggesting concentric fission, resulted.

3. The victims were usually ingested while they were rounded off or sluggish, and, after extrusion, usually failed to resume their activity, although most of them developed contractile vacuoles and some showed tentative amoeboid movements. A few recovered their normal activity and resumed normal life. Amoebae, after extrusion by one amoeba, were often taken up again by other amoebae.

4. In one case an Amoeba vespertilio was observed to chase and enclose an Amoeba limax, but the Amoeba limax subsequently escaped again.

5. The ingested amoebae may easily be mistaken for endogenous buds, but there is less danger of their being mistaken for parasites.

6. No trustworthy evidence was found as to the nature of the stimulus which caused the adoption of these habits, but the question is discussed.

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## EXPLANATION OF PLATES 28 AND 29.

### Plate 28.

Fig. 1.—An Amoeba vespertilio with vesicular nucleus which shows well the meshwork structure of the endosome (*E*) and the clear halo round it. In the cytoplasm is a large vacuole (*vac.*1) containing another amoeba with its nucleus (*n.*1). This second amoeba contains a vacuole (*vac.* 2) which encloses a third amoeba and its nucleus (*n.*2). The third amoeba contains another vacuole (*vac.* 3) which encloses a fourth amoeba and its nucleus (*n.*3). *Ect.*, Ectoplasm. *End.*, Endoplasm.

Fig. 2.—An Amoeba vespertilio with its nucleus (*N*). The amoeba contains food vacuoles and a vacuole (*vac.*1) in which is a second amoeba with its nucleus (*n.*1) and two food vacuoles containing diatoms. This second amoeba contains in a vacuole (*vac.* 2) a third amoeba with its nucleus (*n.*2).

Fig. 3.—A free sphere containing diatoms in food vacuoles (*d*). *n*, nucleus.

Fig. 4.—An amoeba with its nucleus (*N*) and two vacuoles. In one of the latter lies a second amoeba with its nucleus (*n.*3). In the other is a third amoeba with its nucleus (*n.*1), and this again contains a fourth amoeba with its nucleus (*n.*2).

Fig. 5.—An amoeba with its nucleus (*N*) and food vacuoles (*f.b.*), which has ingested one other amoeba with its nucleus (*n*) and food vacuoles (*f.b.*1).

Fig. 6.—A typical free sphere, extruded from an amoeba (compare with the ingested amoeba in fig. 5). The structure of the nucleus is well shown (compare with the nucleus of the outer amoeba in figs. 1, 2, and 4).

### Plate 29.

Fig. 7.—An amoeba with its nucleus (*N*) and a food vacuole (*f.vac.*). It contains three other vacuoles, in two of which two other amoebae lie. One of these, with its nucleus (*n.*1) is free from food bodies; the other, with its nucleus (*n.*2) contains diatoms. The third vacuole contains an amoeba with its nucleus (*n.*3), which itself contains a food vacuole, (*f.vac.*1) and three other amoebae with their nuclei (*n.*4, *n.*5, and *n.*6), the latter being free from food bodies.

Fig. 8.—An amoeba containing two other amoebae in separate vacuoles, one of which is a typical sphere, the other an elongate oval. Both the ingested amoebae contain food bodies.

Fig. 9.—A star-shaped form of Amoeba vespertilio with its nucleus (*N*) and food vacuole (*f.b.*). It contains another amoeba with its nucleus (*n*) and food bodies (*f.b.*1) (cf. Pl. 28, fig. 5).

Fig. 10.—An Amoeba vespertilio with a large food vacuole (*f.vac.*) and an irregularly-shaped amoeba which it has ingested.

Fig. 11.—An amoeba with its nucleus (*N*), which has ingested five other amoebae, the smallest of which are probably A. limax. *n.*1–*n.*5, nuclei of the ingested amoebae.

Fig. 12.—A binucleate amoeba with its two nuclei (*N*,*N*), with food bodies and an ingested amoeba with its nucleus (*n*).